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A PROCESS FOR THE PREPARATION OF ANTIBODIES BY GENETIC ENGINEERING

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(71) Applicant(s)
DR. ANDREAS PLUCKTHUN; ARNE SKERRA

(72) Inventor(s)
DR. ANDREAS PLUCKTHUN; ARNE SKERRA

(74) Attorney or Agent
WATERMARK PATENT & TRADEMARK ATTORNEYS , Locked Bag 5, HAWTHORN VIC 3122

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(57) Claim

1. A gene construct for the preparation of a functional antibody, a functional fragment of an antibody or a fusion protein composed of an antibody domain and other proteins, said gene construct consisting of two or more segments each said segment comprising a gene encoding an individual antibody chain and linked thereto a signal sequence, said two or more segments being coupled to each other so as to form a regulatable operon and so as to enable said genes to undergo simultaneous induction, co-expression and co-secretion in a bacterial host.

2. A process for the in vivo preparation of functional antibodies, functional fragments of the antibodies or fusion proteins composed of antibody domains and other proteins, which comprises coupling genes encoding individual antibody chains each linked to a signal sequence, such that the signal sequence brings about the transport of the antibody chains through the cytoplasmic membrane of a bacterial cell and is then eliminated; bringing about the expression of the genes encoding individual antibody chains to produce functional antibodies, functional fragments of antibodies or fusion proteins composed of antibody domains and other proteins; and isolating the functional antibodies, fragments or fusion proteins from periplasmic space of the bacterium or a medium in which the bacterium is cultured.

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Form 10

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COMPLETE SPECIFICATION

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Name of Applicant : DR. ANDREAS PLUCKTHUN and ARNE SKERRA

Address of Applicant : 6 Veit-Lung-Strasse, D-8033 Planegg and 6 Cheruskerweg D-6200 Wiesbaden, Federal Republic of Germany respectively

Actual Inventor: DR. ANDREAS PLUCKTHUN and ARNE SKERRA

Address for Service : EDWD. WATERS & SONS,
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

A PROCESS FOR THE PREPARATION OF ANTIBODIES BY GENETIC ENGINEERING

The following statement is a full description of this invention, including the best method of performing it known to :- US

Dr. Andreas Plückthun. HOE 87/S 025 Dr. KL/ST
Dipl.-Ing. Arne Skerra,

Description

A process for the preparation of antibodies by genetic engineering

The expression of antibodies in yeast has been described (C.R. Wood, M.A. Boss, J.H. Kenten, J.E. Calvert, N.A. Roberts and J.S. Emtage, Nature 314, 446 (1985)), but only a very small proportion of the expressed protein proved to be functional. In E. coli, to date it has been possible to obtain antibody proteins only in denatured form (M.A. Boss, J.H. Kenten, C.R. Wood and J.S. Emtage, Nucleic Acids Res. 12, 3791 (1984); S. Cabilly, A.D. Riggs, H. Pande, J.E. Shively, W.E. Holmes, M. Rey, L.J. Perry, R. Wetzel and H.L. Heyneker, Proc. Natl. Acad. Sci. U.S.A. 81, 3273 (1984)). The purification of active antibodies or antibody fragments from yeast or other microorganisms has not been disclosed. Attempts at protein folding have to date resulted in only a very small percentage of correctly folded recombinant antibody proteins. In addition, it is difficult to separate the desired functional proteins from undesired and non-functional proteins, which impedes accurate measurement of binding constants, yields of folding, spectral properties and the like, and use in therapy and industry. Optimization of the folding conditions is thus extremely difficult, especially since there are no proven processes and measurement methods for the refolding.

The expression of complete functional antibodies or functional binding domains of antibodies in bacterial expression systems has not been disclosed to date, and the prospects of bringing it about have been assessed pessimistically (S.L. Morrison, Science 229, 1202 (1985), M.A. Boss and C.R. Wood, Immunol. Today 6, 12 (1985)).

Such a system would be very desirable because the genetic

engineering processes have been thoroughly worked out, especially for E. coli, and mass production is facilitated by the rapid growth, which is of considerable economic importance.

Hence the invention relates to the preparation of functional antibodies, functional fragments thereof, or fusion proteins composed of antibody domains and other proteins in bacteria, preferably Gram-negative bacteria, especially in E. coli. The process according to the invention comprises coupling the genes for the individual chains of the antibody molecule or fragment each to a signal sequence which brings about the transport of the polypeptide chains through the cytoplasmic membrane and which can be split off, bringing about the expression of the gene structures, and isolating the functional protein from the periplasmic space or the medium. Preferred embodiments of this invention are explained in detail hereinafter and defined in the patent claims.

The coupling of the genes for the individual chains provided with signal sequences preferably takes place in the manner of a regulatable operon system which brings about simultaneous expression by a common control region. In this way, the individual protein chains are expressed together in approximately the stoichiometric ratio, and are transported into the periplasmic space, where joining to form a functional molecule takes place. Proteins are transported out of the cytoplasm by methods known per se, as are described, for example, in European Patent Specification 0,006,694.

Suitable as control region is every suitable regulatable gene regulatory region, for example lac, tac, trp or synthetic sequences. Particularly preferred are regulatory regions which can be reliably turned off.

The proteins are preferably isolated from the periplasmic

space by exerting a mild osmotic shock on the harvested cells, and subjecting the liquid phase obtained by this to concentration by ultrafiltration or precipitation, for example with salts such as ammonium sulfate.

A "mild" osmotic shock brings about the ejection of the periplasm with, however, the cytoplasmic membrane remaining intact.

The protein concentrate is, expediently after a dialysis, applied to an adsorbent, advantageously in the form of an affinity column, which is loaded with the appropriate antigen or hapten. The antibody or the functional antibody fragment is then obtained by suitable elution, advantageously with the antigen or hapten.

In the eukaryotic cell, antibodies are formed in the lumen of the endoplasmic reticulum - probably with the cooperation of disulfide isomerases, proline cis-trans-isomerases and possibly other enzymes or proteins. It was surprising that the bacterial cell is also able to prepare the two chains in approximately the same stoichiometric amount, to transport the two precursor proteins into the periplasmic space or the medium surrounding it, to eliminate the signal sequences correctly, to fold the globular and soluble domains correctly, to form the intramolecular disulfide linkages, and to associate the two chains to give a heterodimer, because it is regarded as improbable that the bacterial cell is equipped with enzymes which are of this nature or have the same effect Thus, it has surprisingly emerged that, in the case of Gram-negative bacteria, the bacterial periplasm is in this respect functionally equivalent to the lumen of the eukaryotic endoplasmic reticulum.

The process according to the invention has a number of advantages:
apart from the easy and low-cost large-scale bacterial

fermentation already mentioned, there is direct formation of functional proteins, which thus means that the cleavage of fusion proteins, with subsequent isolation of the desired protein or protein fragment, its oxidation or in vitro refolding, are avoided.

Nor have problems with cellular proteases been found in the process according to the invention. It is, after all, known that, because of these cellular proteases, proteins are normally expressed in bacteria in the form of fusion proteins, especially as insoluble inclusion bodies, which, however, is associated with the stated elaborate further processing steps. In contrast, the separation and purification to homogeneity is rapid and straightforward in the process according to the invention.

Thus the invention permits easy access to antibodies, functional fragments thereof and modified antibodies which differ from the natural antibodies by the insertion, elimination and/or exchange of amino acids. Thus, for example, cysteines can be eliminated or replaced by other amino acids in order to suppress undesired folding. It is possible in just the same way to convert a murine into a human antibody, or to introduce other mutations. Thus, besides the possible pharmacological and industrial applications, access is facilitated for research into antibody structure and function and into the fundamentals of enzymatic catalysis (V. Raso and B.D. Stollar, Biochemistry 14, 584 (1975); V. Raso and B.D. Stollar, Biochemistry 14, 591 (1975); A. Tramontano, K.D. Janda and R.A. Lerner, Science 234, 1566 (1986); S.J. Pollack, J.W. Jacobs and P.G. Schultz, Science 234, 1570 (1986); J. Jacobs, P.G. Schultz, R. Sugasawara and M. Powell, J. Am. Chem. Soc. 109, 2174 (1987)).

The invention furthermore permits the application of test systems (assays) directly to the bacterial cell in which the functional antibody is formed, and thus rapid

investigation for possibly mutated antibodies.

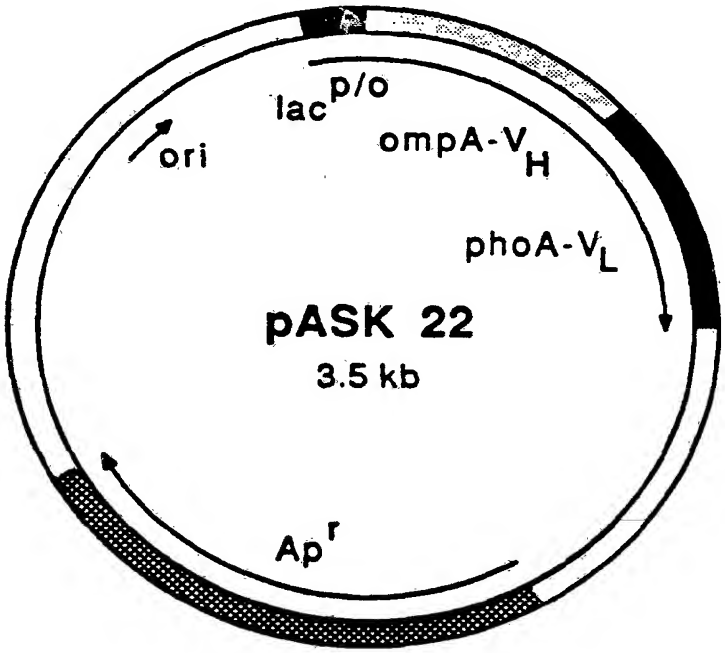
Besides the stated variations in the antibody molecule by altering individual, or a few, amino acids, it is also possible to insert into the antibody gene other gene regions, or to exchange parts for non-critical gene regions. It is possible in this way to couple marker enzymes (M.S. Neuberger, G.T. Williams and R.O. Fox, Nature 312, 604 (1984)), toxins (G. Möller (ed.) "Antibody carriers of drugs and toxins in tumor therapy", Immunol. Rev. 62, Munksgaard, Copenhagen (1982)) or immunoglobulin regions of another class (M.S. Neuberger, G.T. Williams, E.B. Mitchell, S.S. Jouhal, J.G. Flanagan, and T.H. Rabbitts, Nature 314, 268 (1985)) or of another species (P.T. Jones, P.H. Dear, J. Foote, M.S. Neuberger, and G. Winter, Nature 321, 522 (1986)) to the antibody molecule.

The process according to the invention is explained hereinafter taking the example of the variable domains of the phosphorylcholine-binding antibody myeloma protein McPC603. The three-dimensional structure of this mouse immunoglobulin A is known (D.M. Segal, E.A. Padlan, G.H. Cohen, S. Rudikoff, M. Potter and D.R. Davies, Proc. Natl. Acad. Sci. 71, 4298 (1974)). Synthetic genes for the variable light chain V_L and heavy chain V_H were used. Such synthetic genes are proposed in German Offenlegungsschrift 3,715,033 and published European Patent Application 0,290,005 and published Australian Patent Application 15631/88 (Tables 1 and 2 therein). A particularly expedient embodiment of such synthetic genes is shown in the Table, in which the DNA sequence of the complete expression plasmid is depicted, and the genes for the two chains are emphasized by indicating the amino acids. Codons which are rarely used by E. coli were avoided in the construction of these DNA sequences. Furthermore, unique restriction enzyme cleavage sites were incorporated, and account was taken of the secondary structure of the RNA.

In the functional antibody fragment serving as model, each domain has an intramolecular disulfide bridge (from Cys 23 to Cys 94 in V_L and from Cys 22 to Cys 98 in V_H).

There is no disulfide bridge between the chains, nor is there any free cysteine.

The expression vector used, pASK 22, is depicted diagrammatically in the following formula



In it the synthetic genes for the V_L and V_H domains are coupled to gene fragments for the bacterial signal sequence of the outer membrane protein A (ompA) on the one hand, and of alkaline phosphatase (phoA) on the other hand. The genes for the two precursor proteins are located in a synthetic operon-like structure downstream of the lac promoter, which ensures that both genes undergo simultaneous induction, coexpression and cosecretion.

After induction of gene expression, the cells are harvested and exposed to a mild osmotic shock. The liquid

phase obtained by this, which contains the periplasmic proteins, is concentrated by ultrafiltration, dialyzed and applied directly to an affinity column which contains a phosphorylcholine derivative (B. Chesebro and H. Metzger, Biochemistry 11, 766 (1972)) as affinity ligand. Elution with phosphorylcholine results in a homogeneous F_V fragment which is gel electrophoretically homogeneous. It can be deduced from the SDS polyacrylamide gel that the two chains of the purified F_V fragment are present in a molar ratio of 1:1, and the expected molecular weights of the mature proteins (V_H: 13600 D, V_L: 12400 D) are present. To demonstrate correct elimination of the two signal sequences, the six N-terminal amino acids of both chains were sequenced. It emerged that both chains have the correct N-termini for the mature proteins. Thus, both heterologous preproteins have been correctly cleaved by the bacterial signal peptidase, and there is no detectable evidence that imprecise processing or an N-terminal degradation reaction has taken place.

The affinity constant of the recombinant F_V fragment of McPC603 was measured by equilibrium dialysis. The conditions used for this were the same as were applied in the determination of the affinity constant of the natural antibody McPC603 isolated from mouse ascites. The value of $1.21 \pm 0.06 \times 10^5\ M^{-1}$ found for the F_V fragment is identical, within the accuracy of the experiment, to the value of $1.6 \pm 0.4 \times 10^5\ M^{-1}$ reported for the natural antibody. The Scatchard binding plot (Ann. N.Y. Acad. Sci. 51, 660 (1949)) is linear, and extrapolation indicates that approximately 1 mol of hapten is bound per mol of F_V fragment. This confirms that there is only one type of binding site per F_V fragment, and that there are no inactive components present in the isolated protein.

Thus, it has emerged, surprisingly, that it is possible to prepare the F_V fragment of the antibody McPC603 as

completely functional and stable protein in E coli. This demonstrates the functional equivalence of the transport into the periplasm of the bacterial cell with the transport into the lumen of the endoplasmic reticulum of the eukaryotic cell. This equivalence has not been disclosed or even suspected, because the bacterial protein which has hitherto been characterized best and which could be defined as soluble heterodimeric protein in the periplasm - E. coli penicillin acylase - is produced by proteolytic processing from a single-chain precursor in the periplasm (G. Schumacher, D. Sizmann, H. Hang, P. Buckel and A. Böck, Nucleic Acids Res. 14, 5713 (1986)). In addition, it has already been pointed out that, according to present knowledge, bacteria have no enzymes or proteins which might in eukaryotes be involved in the folding.

It was also surprising that the F_V fragment of McPC603 has essentially the same affinity constant for phosphorylcholine as has the intact antibody McPC603 itself. This finding is unexpected because the functionality of F_V fragments is controversial in the literature (J. Sen and S. Beychok, Proteins 1, 256 (1986)). It emerges from this that the functionality can be maintained on modification or even complete deletion of the constant domains.

In contrast to the only method used hitherto for the preparation of F_V fragments, namely by proteolysis of an antibody, there are no problems in the process according to the invention with non-functional, incorrectly folded, incorrectly reassociated or chemically modified proteins. Moreover, the preferred isolation process using an antigen- or hapten-loaded adsorbent is also suitable for purifying those F_V fragments obtained by known processes, because such impurities would either not be bound or not be eluted.

Example 1: Preparation of the plasmid pASK22:

The expression plasmid pASK22 is constructed from the large EcoRI-HindIII fragment of pUC12 (C. Yanisch-Perron, J. Vieira, and J. Messing, Gene 33, 103 (1985)), from fragments of the vectors pIN III-OmpA1 (Y. Masui, J. Coleman and M. Inouye in "Experimental manipulation of gene expression", M. Inouye, ed., Academic Press 1983) and pHI61 (H. Inouye, W. Barnes and J. Beckwith, J. Bacteriol. 149, 434 (1982)) as well as various synthetic DNA fragments, in several stages using methods known per se (T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning, Cold Spring Harbor 1982). The complete DNA sequence of the resulting vector pASK22 is depicted in the Table.

The ligation mixture is used to transform competent E. coli cells, and the latter are selected for ampicillin resistance. The plasmids with the desired gene structure are characterized by restriction analysis and sequencing of the critical junctions.

Example 2: Preparation of the Fv fragment

A culture of the E. coli strain W3110 transformed with pASK22 is cultivated in LB medium containing 100 mg/l ampicillin to an OD_{550} of 0.5. Expression is induced by addition of IPTG to a final concentration of 1mM. After 45 minutes, the cells are harvested by centrifugation at 4000 x g (10 minutes at 4°C). Cell fractionation is carried out by resuspension of the cell pellet in TES buffer (0.2 M tris.HCl, pH 8.0; 0.5 mM EDTA; 0.5 M sucrose) in 10 ml/l of the original culture. The cells are exposed to a mild osmotic shock by addition of 15 ml/l of the original culture of TES buffer which is diluted 1:4 with water and contains 2 mM phosphorylcholine. After the suspension has been incubated on ice for 30 minutes it is centrifuged at 5000 x g for 10 minutes, and the supernatant is subjected to renewed centrifugation at 48000 x g for 15 minutes. The resulting supernatant, which contains all the soluble periplasmic proteins, is

concentrated by ultrafiltration ((R)AMICON YM5 membrane) to a volume of about 2.5 ml/l of the original culture, and is dialyzed against BBS buffer (0.2 M borate/NaOH, pH 8.0; 0.16 M NaCl). This concentrated solution is applied to an affinity column loaded with a phosphorylcholine derivative (B. Chesebro and H. Metzger, loc.cit.) (1-4 ml of solution per ml of bed volume), which is washed with BBS buffer, and the pure Fv fragment is eluted with a solution of 1mM phosphorylcholine in BBS buffer.

Example 3: Equilibrium dialysis

In a dialysis chamber with a volume of 100 µl on each side of the membrane, 50 µl of purified Fv fragment in BBS buffer were placed on one side, and a solution of 50 µl of phosphoryl(methyl-^{14}C)choline (50 mCi/mmol) in BBS buffer was placed on the other side. The concentration of the Fv fragment determined from the OD_{205} of 6.85 was 0.22 mg/ml (R.K. Scopes, Protein Purification - Principles and Practice, Springer-Verlag, New York, 1982, p. 241). After 22 hours at room temperature, equilibrium had been reached, and 20 µl samples of each solution were measured in a scintillation counter (Beckman LS 1801), and the data were subjected to Scatchard plotting (loc. cit.). The affinity constant derived from the gradient of the resulting line is $K_a = 1.21 \pm 0.06 \times 10^5\ M^{-1}$.

TABLE

DNA sequence of pASK22 with the amino acid sequences of ompA-V_H and phoA-V_L

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      GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCA
    1 ---------+---------+---------+---------+---------+---------+ 60
      CGCGGGTTATGCGTTTGGCGGAGAGGGGCGCGCAACCGGCTAAGTAATTACGTCGACCGT

      CGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCT
   61 ---------+---------+---------+---------+---------+---------+ 120
      GCTGTCCAAAGGGCTGACCTTTCGCCCGTCACTCGCGTTGCGTTAATTACACTCAATCGA

      CACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAAT
  121 ---------+---------+---------+---------+---------+---------+ 180
      GTGAGTAATCCGTGGGGTCCGAAATGTGAAATACGAAGGCCGAGCATACAACACACCTTA

      TGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTTCTAGA
  181 ---------+---------+---------+---------+---------+---------+ 240
      ACACTCGCCTATTGTTAAAGTGTGTCCTTTGTCGATACTGGTACTAATGCTTAAAGATCT

      TAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTC
  241 ---------+---------+---------+---------+---------+---------+ 300
      ATTGCTCCCGTTTTTTACTTTTTCTGTCGATAGCGCTAACGTCACCGTGACCGACCAAAG

                          MetLysLysThrAlaIleAlaIleAlaValAlaLeuAlaGlyPhe -

      GCTACCGTAGCGCAGGCCGAAGTTAAACTGGTAGAGTCTGGTGGTGGTCTGGTACAGCCG
  301 ---------+---------+---------+---------+---------+---------+ 360
      CGATGGCATCGCGTCCGGCTTCAATTTGACCATCTCAGACCACCACCAGACCATGTCGGC

      AlaThrValAlaGlnAlaGluValLysLeuValGluSerGlyGlyGlyLeuValGlnPro -

      GGTGGATCCCTGCGTCTGTCTTGCGCTACCTCAGGTTTCACCTTCTCTGACTTCTACATG
  361 ---------+---------+---------+---------+---------+---------+ 420
      CCACCTAGGGACGCAGACAGAACGCGATGGAGTCCAAAGTGGAAGAGACTGAAGATGTAC

      GlyGlySerLeuArgLeuSerCysAlaThrSerGlyPheThrPheSerAspPheTyrMet -

      GAGTGGGTACGTCAGCCCCCGGGTAAACGTCTCGAGTGGATCGCAGCTAGCCGTAACAAA
  421 ---------+---------+---------+---------+---------+---------+ 480
      CTCACCCATGCAGTCGGGGGCCCATTTGCAGAGCTCACCTAGCGTCGATCGGCATTGTTT

      GluTrpValArgGlnProProGlyLysArgLeuGluTrpIleAlaAlaSerArgAsnLys -

      GGTAACAAGTATACCACCGAATACAGCGCTTCTGTTAAAGGTCGTTTCATCGTTTCTCGT
  481 ---------+---------+---------+---------+---------+---------+ 540
      CCATTGTTCATATGGTGGCTTATGTCGCGAAGACAATTTCCAGCAAAGTAGCAAAGAGCA

      GlyAsnLysTyrThrThrGluTyrSerAlaSerValLysGlyArgPheIleValSerArg -

      GACACTAGTCAATCGATCCTGTACCTGCAGATGAATGCATTGCGTGCTGAAGACACCGCT
  541 ---------+---------+---------+---------+---------+---------+ 600
      CTGTGATCAGTTAGCTAGGACATGGACGTCTACTTACGTAACGCACGACTTCTGTGGCGA

      AspThrSerGlnSerIleLeuTyrLeuGlnMetAsnAlaLeuArgAlaGluAspThrAla -
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                ATCTACTACTGCGCGCGTAACTACTATGGCAGCACTTGGTACTTCGACGTTTGGGGTGCA
          601   ---------+---------+---------+---------+---------+---------+  660
                TAGATGATGACGCGCGCATTGATGATACCGTCGTGAACCATGAAGCTGCAAACCCCACGT

                IleTyrTyrCysAlaArgAsnTyrTyrGlySerThrTrpTyrPheAspValTrpGlyAla -

                GGTACCACCGTTACCGTTTCTTCTTGATAACATGGAGAAAATAAAGTGAAACAAAGCACT
          661   ---------+---------+---------+---------+---------+---------+  720
                CCATGGTGGCAATGGCAAAGAAGAACTATTGTACCTCTTTTATTTCACTTTGTTTCGTGA

                GlyThrThrValThrValSerSerEnd                    MetLysGlnSerThr -

                ATTGCACTGGCACTCTTACCGTTACTGTTTACCCCTGTGACAAAAGCCGATATCGTTATG
          721   ---------+---------+---------+---------+---------+---------+  780
                TAACGTGACCGTGAGAATGGCAATGACAAATGGGGACACTGTTTTCGGCTATAGCAATAC

                IleAlaLeuAlaLeuLeuProLeuLeuPheThrProValThrLysAlaAspIleValMet -

                ACCCAGTCTCCGAGCTCTCTGTCTGTATCTGCAGGTGAACGTGTTACCATGTCTTGCAAA
          781   ---------+---------+---------+---------+---------+---------+  840
                TGGGTCAGAGGCTCGAGAGACAGACATAGACGTCCACTTGCACAATGGTACAGAACGTTT

                ThrGlnSerProSerSerLeuSerValSerAlaGlyGluArgValThrMetSerCysLys -

                TCTTCTCAGTCTCTGCTGAACTCTGGTAACCAGAAAAACTTCCTGGCGTGGTATCAGCAA
          841   ---------+---------+---------+---------+---------+---------+  900
                AGAAGAGTCAGAGACGACTTGAGACCATTGGTCTTTTTGAAGGACCGCACCATAGTCGTT

                SerSerGlnSerLeuLeuAsnSerGlyAsnGlnLysAsnPheLeuAlaTrpTyrGlnGln -

                AAGCCTGGCCAACCGCCGAAACTGCTGATCTACGGTGCGTCGACCCGTGAATCTGGTGTT
          901   ---------+---------+---------+---------+---------+---------+  960
                TTCGGACCGGTTGGCGGCTTTGACGACTAGATGCCACGCAGCTGGGCACTTAGACCACAA

                LysProGlyGlnProProLysLeuLeuIleTyrGlyAlaSerThrArgGluSerGlyVal -

                CCGGACCGTTTTACCGGTAGCGGTAGCGGTACCGACTTCACTCTGACCATCTCTTCTGTA
          961   ---------+---------+---------+---------+---------+---------+ 1020
                GGCCTGGCAAAATGGCCATCGCCATCGCCATGGCTGAAGTGAGACTGGTAGAGAAGACAT

                ProAspArgPheThrGlySerGlySerGlyThrAspPheThrLeuThrIleSerSerVal -

                CAGGCTGAAGATCTGGCTGTTTACTACTGTCAAAACGACCACTCTTACCCGCTGACCTTT
         1021   ---------+---------+---------+---------+---------+---------+ 1080
                GTCCGACTTCTAGACCGACAAATGATGACAGTTTTGCTGGTGAGAATGGGCGACTGGAAA

                GlnAlaGluAspLeuAlaValTyrTyrCysGlnAsnAspHisSerTyrProLeuThrPhe -

                GGCGCCGGCACCAAACTGGAACTGAAGCGCGCTTGATAAGCTTGGCACTGGCCGTCGTTT
         1081   ---------+---------+---------+---------+---------+---------+ 1140
                CCGCGGCCGTGGTTTGACCTTGACTTCGCGCGAACTATTCGAACCGTGACCGGCAGCAAA

                GlyAlaGlyThrLysLeuGluLeuLysArgAlaEnd

                TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATC
         1141   ---------+---------+---------+---------+---------+---------+ 1200
                ATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCGTGTAG

                CCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGT
         1201   ---------+---------+---------+---------+---------+---------+ 1260
                GGGGAAAGCGGTCGACCGCATTATCGCTTCTCCGGGCGTGGCTAGCGGGAAGGGTTGTCA
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      TGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCG
1261  ---------+---------+---------+---------+---------+---------+ 1320
      ACGCGTCGGACTTACCGCTTACCGCGGACTACGCCATAAAAGAGGAATGCGTAGACACGC

      GTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA
1321  ---------+---------+---------+---------+---------+---------+ 1380
      CATAAAGTGTGGCGTATACCACGTGAGAGTCATGTTAGACGAGACTACGGCGTATCAATT

      GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG
1381  ---------+---------+---------+---------+---------+---------+ 1440
      CGGTCGGGGCTGTGGGCGGTTGTGGGCGACTGCGCGGGACTGCCCGAACAGACGAGGGCC

      CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCAC
1441  ---------+---------+---------+---------+---------+---------+ 1500
      GTAGGCGAATGTCTGTTCGACACTGGCAGAGGCCCTCGACGTACACAGTCTCCAAAAGTG

      CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTA
1501  ---------+---------+---------+---------+---------+---------+ 1560
      GCAGTAGTGGCTTTGCGCGCTCTGCTTTCCCGGAGCACTATGCGGATAAAAATATCCAAT

      ATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCG
1561  ---------+---------+---------+---------+---------+---------+ 1620
      TACAGTACATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGC

      GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAAT
1621  ---------+---------+---------+---------+---------+---------+ 1680
      CTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTA

      AACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCC
1681  ---------+---------+---------+---------+---------+---------+ 1740
      TTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTGTAAAGG

      GTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAA
1741  ---------+---------+---------+---------+---------+---------+ 1800
      CACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTT

      CGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAAC
1801  ---------+---------+---------+---------+---------+---------+ 1860
      GCGACCACTTTCATTTTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCTTG

      TGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGA
1861  ---------+---------+---------+---------+---------+---------+ 1920
      ACCTAGAGTTGTCGCCATTCTAGGAACTCTCAAAAGCGGGGCTTCTTGCAAAAGGTTACT

      TGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAG
1921  ---------+---------+---------+---------+---------+---------+ 1980
      ACTCGTGAAAATTTCAAGACGATACACCGCGCCATAATAGGGCATAACTGCGGCCCGTTC

      AGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCA
1981  ---------+---------+---------+---------+---------+---------+ 2040
      TCGTTGAGCCAGCGGCGTATGTGATAAGAGTCTTACTGAACCAACTCATGAGTGGTCAGT
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      CAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCA
2041  ---------+---------+---------+---------+---------+---------+ 2100
      GTCTTTTCGTAGAATGCCTACCGTACTGTCATTCTCTTAATACGTCACGACGGTATTGGT

      TGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAA
2101  ---------+---------+---------+---------+---------+---------+ 2160
      ACTCACTATTGTGACGCCGGTTGAATGAAGACTGTTGCTAGCCTCCTGGCTTCCTCGATT

      CCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGC
2161  ---------+---------+---------+---------+---------+---------+ 2220
      GGCGAAAAAACGTGTTGTACCCCCTAGTACATTGAGCGGAACTAGCAACCCTTGGCCTCG

      TGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAA
2221  ---------+---------+---------+---------+---------+---------+ 2280
      ACTTACTTCGGTATGGTTTGCTGCTCGCACTGTGGTGCTACGGACATCGTTACCGTTGTT

      CGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAG
2281  ---------+---------+---------+---------+---------+---------+ 2340
      GCAACGCGTTTGATAATTGACCGCTTGATGAATGAGATCGAAGGGCCGTTGTTAATTATC

      ACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCT
2341  ---------+---------+---------+---------+---------+---------+ 2400
      TGACCTACCTCCGCCTATTTCAACGTCCTGGTGAAGACGCGAGCCGGGAAGGCCGACCGA

      GGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCAC
2401  ---------+---------+---------+---------+---------+---------+ 2460
      CCAAATAACGACTATTTAGACCTCGGCCACTCGCACCCAGAGCGCCATAGTAACGTCGTG

      TGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAA
2461  ---------+---------+---------+---------+---------+---------+ 2520
      ACCCCGGTCTACCATTCGGGAGGGCATAGCATCAATAGATGTGCTGCCCCTCAGTCCGTT

      CTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGT
2521  ---------+---------+---------+---------+---------+---------+ 2580
      GATACCTACTTGCTTTATCTGTCTAGCGACTCTATCCACGGAGTGACTAATTCGTAACCA

      AACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAAT
2581  ---------+---------+---------+---------+---------+---------+ 2640
      TTGACAGTCTGGTTCAAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTA

      TTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTG
2641  ---------+---------+---------+---------+---------+---------+ 2700
      AATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCAC

      AGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
2701  ---------+---------+---------+---------+---------+---------+ 2760
      TCAAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAG

      CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGG
2761  ---------+---------+---------+---------+---------+---------+ 2820
      GAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATGGTCGCCACC
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                TTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAG
2821            ---------+---------+---------+---------+---------+---------+ 2880
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                CGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACT
2881            ---------+---------+---------+---------+---------+---------+ 2940
                GCGTCTATGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGA

                CTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTG
2941            ---------+---------+---------+---------+---------+---------+ 3000
                GACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCAC

                GCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC
3001            ---------+---------+---------+---------+---------+---------+ 3060
                CGCTATTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCG

                GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCG
3061            ---------+---------+---------+---------+---------+---------+ 3120
                CCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCTTGCTGGATGTGGC

                AACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG
3121            ---------+---------+---------+---------+---------+---------+ 3180
                TTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCC

                CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAG
3181            ---------+---------+---------+---------+---------+---------+ 3240
                GCCTGTCCATAGGCCATTCGCCGTCCCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTC

                GGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTC
3241            ---------+---------+---------+---------+---------+---------+ 3300
                CCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAG

                GATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCT
3301            ---------+---------+---------+---------+---------+---------+ 3360
                CTAAAAACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGA

                TTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCC
3361            ---------+---------+---------+---------+---------+---------+ 3420
                AAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGG

                CTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCC
3421            ---------+---------+---------+---------+---------+---------+ 3480
                GACTAAGACACCTATTGGCATAATGGCGGAAACTCACTCGACTATGGCGAGCGGCGTCGG

                GAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA
3481            ---------+---------+---------+---------+--- 3523
                CTTGCTGGCTCGCGTCGCTCAGTCACTCGCTCCTTCGCCTTCT
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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A gene construct for the preparation of a functional antibody, a functional fragment of an antibody or a fusion protein composed of an antibody domain and other proteins, said gene construct consisting of two or more segments each said segment comprising a gene encoding an individual antibody chain and linked thereto a signal sequence, said two or more segments being coupled to each other so as to form a regulatable operon and so as to enable said genes to undergo simultaneous induction, co-expression and co-secretion in a bacterial host.

2. A process for the in vivo preparation of functional antibodies, functional fragments of the antibodies or fusion proteins composed of antibody domains and other proteins, which comprises coupling genes encoding individual antibody chains each linked to a signal sequence, such that the signal sequence brings about the transport of the antibody chains through the cytoplasmic membrane of a bacterial cell and is then eliminated; bringing about the expression of the genes encoding individual antibody chains to produce functional antibodies, functional fragments of antibodies or fusion proteins composed of antibody domains and other proteins; and isolating the functional antibodies, fragments or fusion proteins from periplasmic space of the bacterium or a medium in which the bacterium is cultured.

3. A process for the in vivo preparation of a functional antibody, a functional fragment of the antibody or a fusion protein comprising an antibody domain, comprising placing on a single plasmid a first DNA sequence consisting of:

(a) a first nucleotide sequence that encodes an antibody heavy chain or a functional fragment thereof, and

(b) a first signal sequence derived from a prokaryote, and

a second DNA sequence consisting of:

(a) a second nucleotide sequence that encodes an antibody light chain or a functional fragment thereof, and

(b) a second signal sequence derived from the prokaryote;

bringing about expression of the first and second nucleotide sequences in a bacterium to produce a functional antibody, a functional fragment of the antibody or a fusion protein comprising an antibody domain, and isolating the functional antibody, functional fragment or the fusion protein from periplasmic space of the bacterium or from a medium in which the bacterium is cultured.

4. The process as claimed in claims 2 or 3, wherein the bacterium is a Gram-negative bacterium.

5. The process as claimed in claim 4, wherein the bacterium is E. coli.

6. The process as claimed in claims 2 - 5, wherein the genes are coupled in the form of a regulatable operon system.

7. The process as claimed in one or more of the preceding claims, wherein, in order to isolate the functional protein from the periplasmic space, the bacteria which have been separated off are exposed to an osmotic shock such that the periplasm is ejected but the cytoplasm remains in tact, the liquid phase obtained by this is enriched by centrifugation, and the desired proteins are obtained from this concentrate.

8. The process as claimed in one or more of the preceding claims, wherein the solution containing the functional protein is applied to an absorbent loaded with the appropriate antigen or hapten, and the desired proteins are isolated by elution with a solution containing this antigen or hapten.

9. A functional antibody, a functional fragment of an antibody or a fusion protein composed of an antibody domain and other proteins prepared from a gene construct as claimed in claim 1.

10. A functional antibody, a functional fragment of an antibody or a fusion protein composed of an antibody domain and other proteins prepared according to the process of any one of claims 2 - 8.

DATED this 11th day of March 1992.
DR ANDREAS PLUCKTHUN and ARNE SKERRA

WATERMARK PATENT & TRADEMARK ATTORNEYS
THE ATRIUM
290 BURWOOD ROAD
HAWTHORN VICTORIA 3122
AUSTRALIA

DBM/KJS/CH (DOC.10) AU2761788.WPC